Calcium signalling in lymphocyte activation and disease

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Abstract | Calcium signals in cells of the immune system participate in the regulation of cell differentiation, gene transcription and effector functions. An increase in intracellular levels of calcium ions (Ca^{2+}) results from the engagement of immunoreceptors, such as the T-cell receptor, B-cell receptor and Fc receptors, as well as chemokine and co-stimulatory receptors. The major pathway that induces an increase in intracellular Ca^{2+} levels in lymphocytes is through store-operated calcium entry (SOCE) and calcium-release-activated calcium (CRAC) channels. This Review focuses on the role of Ca^{2+} signals in lymphocyte functions, the signalling pathways leading to Ca^{2+} influx, the function of the recently discovered regulators of Ca^{2+} influx (STIM and ORAI), and the relationship between Ca^{2+} signals and diseases of the immune system.

Calcium ions (Ca^{2+}) function as a universal second messenger in virtually all eukaryotic cells, including cells of the immune system. Ca^{2+} signals are crucial for the proper activation of lymphocytes, some aspects of their differentiation and effector functions, such as the formation of a stable synapse between T cells and antigen-presenting cells (APCs), and vesicle exocytosis in cytotoxic T cells (CTLs). An influx of Ca^{2+} into the cytosol occurs following the engagement of immunoreceptors on the cell surface, for example by antigen binding to the T-cell receptor (TCR) or the B-cell receptor (BCR), or by binding of antigen–antibody complexes to the low-affinity Fc receptor for IgE (FcεRII) on mast cells or to Fc receptors for IgG (FcγRs) on natural killer (NK) cells, dendritic cells (DCs) or macrophages (FIG. 1, TABLE 1). In lymphocytes, store-operated calcium entry (SOCE) through calcium-release-activated calcium (CRAC) channels is the main mechanism to increase intracellular Ca^{2+} concentrations, and it is essential for the activation of and cytokine gene expression by T and B cells (reviewed in REFS 1–3). In the absence of sustained Ca^{2+} influx through CRAC channels, lymphocyte activation, proliferation and effector functions are severely compromised, as demonstrated by the existence of rare but very instructive immunodeficiency diseases in humans[4–6]. With the discovery of two molecules, stromal interaction molecule 1 (STIM1) and ORAI1 (also known as CRACM1 or TMEM142A), in the past 2 years[7], our understanding of how Ca^{2+} signals in lymphocytes are regulated has increased significantly; their function is described in detail in this Review.

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Role of Ca^{2+} signals in lymphocytes

Ca^{2+} signals have been described in various cells of the immune system, including T and B cells, NK cells, mast cells, DCs, monocytes and macrophages, in which they contribute to the cells' activation, effector functions, gene expression or differentiation. The major Ca^{2+} signalling pathways and functions are summarized in TABLE 1 (see supplementary information S1 (table) for a fully referenced version). This Review, however, focuses on the role of Ca^{2+} signals in T and B cells.

Ca^{2+} signals were recognized to be important for RNA synthesis and mitotic cell division in leukocytes and thymocytes about four decades ago[8,9]. The subsequent analysis of mutant T cells that were deficient for Ca^{2+} influx showed that the lack of TCR-mediated Ca^{2+} signals results in impaired interleukin-2 (IL-2) production and T-cell proliferation *in vitro*[10] and defective T-cell-mediated immune responses *in vivo*[4–6,11–13]. Since then the role of Ca^{2+} in T-cell activation has been studied in great detail (reviewed in REFS 1,14–17). As is discussed here, the main source of Ca^{2+} signals in T cells is through SOCE and the activation of CRAC channels. The strength and duration of the Ca^{2+} signal generated by this mechanism determines, in concert with other signalling pathways, the T-cell response to activation. To illustrate the different roles that Ca^{2+} signals have in T cells, I distinguish between the short-term and long-term consequences of an increase in intracellular Ca^{2+} levels.

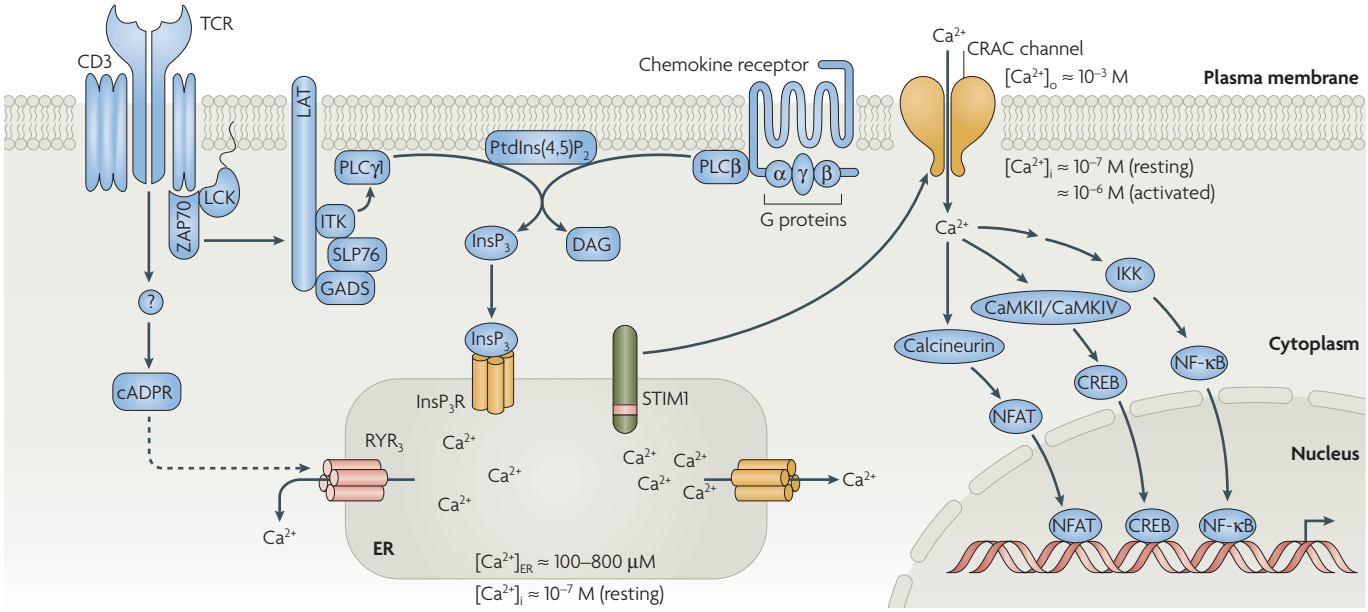


Figure 1 | **Store-operated calcium entry (SOCE) in T cells.** In resting T cells, a steep gradient in Ca^{2+} concentrations exists between the cytoplasm and the extracellular space, as well as between the cytoplasm and the lumen of the endoplasmic reticulum (ER). The intracellular Ca^{2+} concentrations in T cells is tightly regulated and kept between ~100 nM in resting cells and ~1 μM following T-cell receptor (TCR) stimulation. Antigen recognition through the TCR results in the activation of protein tyrosine kinases, such as LCK and ZAP70 (ζ-chain-associated protein kinase of 70 kDa), which initiate phosphorylation events of adaptor proteins, such as SLP76 (SRC-homology-2-domain-containing leukocyte protein of 76 kDa) and LAT (linker for activation of T cells). This leads to the recruitment and activation of the TEC kinase ITK (interleukin-2-inducible T-cell kinase) and phospholipase Cγ1 (PLCγ1). Similarly, binding of G-protein-coupled chemokine receptors results in the activation of PLCβ. PLCβ and PLCγ1 catalyse the hydrolysis of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) to inositol-1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). $InsP_3$ binds to and opens $InsP_3$ receptors ($InsP_3Rs$) in the membrane of the ER, resulting in the release of Ca^{2+} from intracellular Ca^{2+} stores. A decrease in the Ca^{2+} content of the ER is 'sensed' by stromal interaction molecule 1 (STIM1), which in turn activates calcium-release-activated calcium (CRAC) channels in the plasma membrane. Ca^{2+} influx though CRAC channels and elevated intracellular Ca^{2+} concentration activate Ca^{2+}-dependent enzymes, such as calcineurin, and thereby transcription factors, such as NFAT (nuclear factor of activated T cells), NF-κB (nuclear factor-κB) and CREB (cyclic-AMP-responsive-element-binding protein). cADPR, cyclic ADP ribose; CaMK, calmodulin-dependent kinase; GADS, growth-factor-receptor-bound-protein-2-related adaptor protein; IKK, inhibitor of NF-κB kinase; RYR_3, ryanodine receptor 3.

Short-term functions of Ca^{2+} signals. The regulation of lymphocyte motility and immunological synapse formation are Ca^{2+}-dependent processes. Several studies using *in vitro* and *in vivo* imaging of T cells have shown that an increase in intracellular Ca^{2+} concentration results in reduced mobility and rounding of otherwise polymorphic T cells[18,19], whereas inhibition of the Ca^{2+} increase, for example by using intracellular Ca^{2+} buffers, prevents these processes. Observations in mature T cells and thymocytes suggest that an increase in intracellular Ca^{2+} concentration is both necessary and sufficient to deliver a 'stop' signal to T cells[18–20]. This stop signal seems to sustain the interaction between a $CD4^+$ T cell and an APC and the formation of the immunological synapse. When intracellular Ca^{2+} levels are buffered or Ca^{2+} influx is prevented, a stable immunological synapse is not formed[18,20]. An immunological synapse is also formed between CTLs and their target cells, such as virus-infected cells and tumour cells, to ensure the localized release of perforin- and granzyme-containing lytic granules[21]. Synapse formation is accompanied by a rise in intracellular Ca^{2+} concentration in CTLs, which is required for granule exocytosis and target-cell killing[22,23]. But whereas the contact between $CD4^+$ T cells and APCs and the associated increase in Ca^{2+} levels have to last for several hours to induce changes in gene expression, CTL-mediated cytotoxicity, including immunological synapse formation, the release of lytic granules and the first signs of cell death in the target cell, is fast and occurs within the first 5 minutes of target-cell recognition[22].

Long-term functions of Ca^{2+} signals. Ca^{2+}-dependent transcriptional responses shape the medium- to long-term outcome of T-cell and B-cell activation because they influence gene expression patterns (such as cytokine expression profiles), which determine lymphocyte effector functions, the regulation of a state of unresponsiveness (known as anergy), the differentiation of naive T cells into T helper 1 (T_H1) or T_H2 cells and the development of immature T cells. It should be emphasized that the long-term developmental and differentiation programmes, although initiated with the help of Ca^{2+} signals, do not require long-term increases in intracellular Ca^{2+} levels, but are imparted by changes in Ca^{2+}-dependent gene expression and epigenetic changes.

Immunological synapse
A large junctional structure that is formed at the cell surface between a T cell and an antigen-presenting cell (APC); it consists of molecules required for adhesion and signalling. This structure is important in establishing T-cell adhesion and polarity, is influenced by the cytoskeleton and transduces highly controlled secretory signals, thereby allowing the directed release of cytokines or lytic granules towards the APC or target cell.

Table 1 | **Ca^{2+} signalling pathways and functions in cells of the immune system***

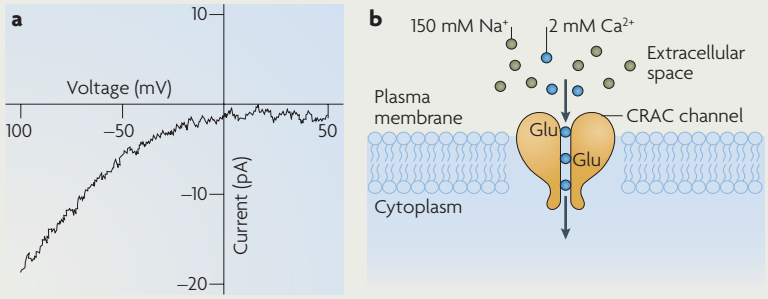
Cell type	Ca^{2+} pathway and/or Ca^{2+} channel	Modulators	Functions; associated disease
T cells, T_{Reg} cells and NKT cells	• TCR→ PLCγ1 → $InsP_3R$ → STIM1 → CRAC channel (ORAI1) • Chemokine receptor → PLCβ → $InsP_3R$‡ → CRAC channel‡ • TCR → cADPR → RYR_3 → CRAC channel (TRPM2)‡ • ATP → P2X and P2Y receptors • L-type voltage-gated Ca^{2+} channels‡ • CD38	• IK1 (positive) • Kv1.3 (positive) • TRPM4 (negative)	T-cell activation, anergy, gene expression, motility, synapse formation, cytotoxicity, development and differentiation; SCID, autoimmunity
B cells	• BCR → PLCγ2 → $InsP_3R$ → STIM1 → CRAC channel • BCR → cADPR → RYR_3 → CRAC channel • CD19 • TRPC1, TRPC7 • $InsP_3R$ (plasma membrane) • CD38	• CD20 (positive) • CD81 (positive) • CD22 (negative) • PIRB → SHP1, SHP2 (negative) • FcγRIIB→ SHIP (negative)	B-cell activation and maturation; XLA, CVID, autoimmunity
Mast cells	• FcεR → PLCγ1 → $InsP_3R$ → STIM1 → CRAC channel • FcεR → SPHK1 → store depletion • ATP → P2X and P2Y receptors	• IRp60 (CD300a) (negative)	Degranulation, histamine release
NK cells	• FcγRIIIA (CD16) → PLCγ2 → $InsP_3R$ → CRAC channel‡ • NKG2D → DAP10/GRB2-VAV1/p85 → PLCγ‡ → $InsP_3R$‡ • NKRP1C (CD161) • CD38 • 2B4 (CD244)	• NKRP1B (CD161) (negative) • KIR2DL1 (CD158a) (negative) • KIR3DL1 (CD158E1) (negative)	Cytolytic activity in response to target-cell recognition
DCs	• Chemokine receptor (CCR7, CXCR4) → PLCβ → $InsP_3R$ → CRAC channel • ATP → P2X and P2Y receptors • CD38	None known	Maturation of DCs from myeloid progenitors; expression of MHC class II and co-stimulatory molecules; migration of immature DCs to secondary lymphoid organs
Macrophages	• FcγR → PLCγ → $InsP_3R$ → CRAC channel • Chemokine receptors (CCR5, CXCR4) → CRAC channel • [LPS → TLR4] or [P3C → TLR2] → PLCγ → $InsP_3R$‡ → CRAC channel‡	None known	Pro-inflammatory cytokine gene expression, iNOS and TNF expression, phagocytosis (?)
Neutrophils	• FcγRIIa/FcγRIIIb → PLCγ → $InsP_3R$ → CRAC channel‡ • IL-8 → PLCγ → $InsP_3R$ → CRAC channel‡	None known	Phagocytosis

*See supplementary information S1 (table) for a fully referenced version. ‡These molecules and channels may be involved in the generation of Ca^{2+} signals but direct evidence is missing. BCR, B-cell receptor; cADPR, cyclic ADP ribose; CCR, CC-chemokine receptor; CRAC, calcium-release-activated calcium; CVID, common variable immunodeficiency; CXCR4, CXC-chemokine receptor 4; DC, dendritic cell; FcεR, Fc receptor for IgE; FcγR, Fc receptor for IgG; GRB2, growth-factor-receptor-bound protein 2; IK1, intermediate conductance calcium-activated potassium channel 1; IL-8, interleukin-8; iNOS, inducible nitric-oxide synthase; $InsP_3R$, inositol-1,4,5-triphosphate receptor; IRp60, inhibitory receptor protein 60 kDa; KIR, killer-cell immunoglobulin-like receptor; Kv1.3, voltage-gated potassium channel Kv1.3; LPS, lipopolysaccharide; NKG2D, natural-killer group 2, member D; NKRP1, NK-cell receptor protein 1; NKT, NK T cells; P3C, tripalmitoyl-S-glycerylcysteine; PIRB, paired immunoglobulin-like receptor B; PLC, phospholipase C; RYR_3, ryanodine receptor 3; SCID, severe combined immunodeficiency; SHIP, SRC homology 2 (SH2)-domain-containing inositol-5-phosphatase; SHP, SH2-domain-containing protein tyrosine phosphatase; SPHK1, sphingosine kinase 1; STIM1, stromal interaction molecule 1; TCR, T-cell receptor; TLR, Toll-like receptor; TNF, tumour-necrosis factor; T_{Reg}, regulatory T; TRPC, transient receptor potential channel, subfamily C; TRPM2, transient receptor potential channel, subfamily M, member 2; XLA, X-linked agammaglobulinaemia.

Ca^{2+}-dependent gene expression requires sustained increases in intracellular Ca^{2+} concentration for several hours. The strength and the duration of the Ca^{2+} signal emanating from the TCR and store-operated CRAC channels determines the pattern of Ca^{2+}-dependent gene expression because the signalling molecules and transcription factors involved in decoding the rise in Ca^{2+} levels and the genes they regulate have different requirements for activation. Ca^{2+} signals result in the activation of transcription factors, such as CREB (cyclic-AMP-responsive-element-binding protein), MEF2 (myocyte enhancer factor 2A) and NFAT (nuclear factor of activated T cells). Probably the best studied Ca^{2+}-responsive signalling pathway in T cells involves the phosphatase calcineurin, which dephosphorylates NFAT proteins following an increase in intracellular Ca^{2+} concentration and leads to its nuclear translocation[14,15,24]. NFAT is regulated in a highly dynamic manner by Ca^{2+} levels because a decrease in intracellular Ca^{2+} levels results in the almost instantaneous phosphorylation and export of NFAT from the nucleus. As a consequence, NFAT-dependent gene transcription is only poorly activated in response to a single pulse of high intracellular Ca^{2+} levels but requires prolonged elevation of Ca^{2+} levels[25]. This is in contrast to another transcription factor, nuclear factor-κB (NF-κB), or the JUN N-terminal kinase (JNK) and its substrate ATF2 (activating transcription factor 2), for which a transient increase in intracellular Ca^{2+} concentration is sufficient for activation and subsequent target gene expression[25]. The role of the Ca^{2+}–calcineurin–NFAT signalling pathway for gene transcription in T cells has been emphasized by studies investigating the effects of blocking calcineurin activity with the immunosuppressants cyclosporin A and FK506 (REFS 12,26,27) or using T cells with genetic defects in Ca^{2+} influx[12,28]. Either abrogated calcineurin activity or a lack of Ca^{2+} influx resulted in the severely impaired expression of cytokines and several hundred other genes[12,26,27,29] (reviewed in REFS 14,30). These studies also indicated that Ca^{2+} signals exert both stimulatory and inhibitory effects on gene expression[12,26].

NFATs
(Nuclear factors of activated T cells). A family of transcription factors consisting of five members: NFAT1 (also known as NFATc2), NFAT2 (also known as NFATc1), NFAT3 (also known as NFATc4), NFAT4 (also known as NFATc3) and NFAT5 (also known as TonEBP). Except for NFAT5, all NFAT proteins are regulated by calcium signals. In addition to their role in T cells, NFAT proteins have regulatory roles in many organs, including the central nervous system, cardiovascular system, kidney, bone and skeletal muscle.

Box 1 | **Biophysical properties of the CRAC channel**



Calcium-release-activated calcium (CRAC) channels are the main source of Ca^{2+} influx in many cells of the immune system and they are essential for T-cell activation and expression of many genes (reviewed in REFS 1–3). CRAC channel currents (I_{CRAC}) were first described in mast cells[47] and T cells[48] around 15 years ago based on their electrophysiological properties from patch-clamp recordings. An inwardly rectifying current to voltage relationship in whole cell patch-clamp recordings is a characteristic feature of the CRAC channel (see Figure part **a**). Depletion of Ca^{2+} stores is essential for CRAC channel activation and is achieved by either immunoreceptor binding or passively using intracellular Ca^{2+} chelators (such as BAPTA), the drug inhibitor thapsigargin, which blocks the Ca^{2+} ATPase (SERCA) responsible for re-uptake of Ca^{2+} into the endoplasmic reticulum (ER), or Ca^{2+} ionophores, which release Ca^{2+} from ER stores. Passive store depletion induces the same store-operated calcium entry (SOCE) as, for example, signals emanating from the T-cell receptor, indicating that store depletion is necessary and sufficient to activate SOCE. Before the recent discovery of ORAI1 as part of the CRAC channel pore (see main text and REFS 13,78,79), the channel could only be identified functionally by a set of distinct biophysical properties. These properties include a very high selectivity for Ca^{2+} over Na^+ (owing to two glutamate residues in the pore of the CRAC channel[80,83,84]; see Figure, part **b**), an extremely low unitary conductance of less than 1 pS and rapid Ca^{2+}-dependent inactivation (reviewed in REFS 1–3). Part **a** of the Figure is reproduced with permission from REF. 6 © (2005) Rockefeller University Press.

Whether Ca^{2+}-dependent transcriptional responses result in lymphocyte activation or inhibition also depends on the context in which T and B cells are stimulated. Sustained Ca^{2+} signals following TCR or BCR engagement in the absence of co-stimulatory signals were shown to induce anergy in T and B cells, a state in which these cells become unresponsive to subsequent antigenic stimulation[31]. In T cells, unresponsiveness induced in this way is characterized by the transcription of around 200 anergy-associated genes, among these are the E3 ubiquitin ligases *ITCH* (itchy homologue E3 ubiquitin protein ligase), *CBL-B* (casitas B-lineage lymphoma B) and *GRAIL* (gene related to anergy in lymphocytes), which function as negative regulators of T-cell activation by targeting signal transduction molecules, such as protein kinase Cθ (PKCθ) and phospholipase Cγ1 (PLCγ1) for degradation[29,32,33].

During T_H-cell differentiation, naive $CD4^+$ T cells on encounter with antigen become interferon-γ (IFNγ)-producing and IL-4-producing T_H1 and T_H2 cells, respectively, a process that depends on the cytokine milieu and the activity of important transcription factors such as T-bet and GATA3 (GATA-binding protein 3). The strength of the TCR signal also influences the fate of naive T cells because strong TCR agonists, which induce a robust Ca^{2+} signal and IL-2 production, tend to induce T_H1-cell differentiation[34], whereas weak agonists favour a T_H2-cell response[35–37]. Furthermore, TCR-induced Ca^{2+} signals were shown to be lower in T_H2 cells than in T_H1 cells or naive T cells, a phenomenon that might help T_H1 and T_H2 cells remain committed to their cell fate[37,38].

The role of Ca^{2+} signals in lymphocyte development is less clearly defined, as so far there are no genetic models available that target molecules specific for SOCE in developing T and B cells. Several lines of evidence support a potential role for Ca^{2+} influx in T-cell development. First, engagement of the pre-TCR expressed on $CD4^-CD8^-$ double negative (DN) thymocytes causes an increase in intracellular Ca^{2+} concentration, which is likely to contribute to the survival and proliferation signal required for the transition of immature thymocytes from the DN to the $CD4^+CD8^+$ double positive (DP) stage[39]. Second, mice deficient for signalling molecules involved in generating Ca^{2+} signals downstream of the pre-TCR, such as SLP76 (SRC homology 2 (SH2)-domain-containing leukocyte protein of 76 kDa), LAT (linker for activation of T cells) and ITK (IL-2-inducible T-cell kinase), show a block in T-cell development[40–43]. Third, mice deficient for some isoforms of important downstream effectors of Ca^{2+} signals, such as calcineurin and NFAT, show impaired T-cell development (reviewed in REF. 24). Fourth, positive selection of T cells was shown to depend on Ca^{2+} oscillations in thymocytes[20].

Taken together, Ca^{2+} signals participate in the regulation of various effector functions and cell-fate decisions in lymphocytes. Consequently, abnormal Ca^{2+} signals result in several immunodeficiency and autoimmune disorders, as discussed later.

Mechanisms of Ca^{2+} signalling in lymphocytes

TCR signals and SOCE. The main intracellular signalling events that induce an increase in intracellular Ca^{2+} concentration are shared by practically all cell types of the immune system. Antigen binding to immunoreceptors, such as the TCR, BCR and FcγRs, leads, via activation of several layers of protein kinases, to the activation of PLC — PLCγ1 in T cells and mast cells, and PLCγ2 in B cells (reviewed in REFS 44–46) (FIG. 1). The resulting hydrolysis of phosphatidylinositol-3,4-bisphosphate ($PtdIns(3,4)P_2$) to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($InsP_3$) leads to the binding of $InsP_3$ to the $InsP_3$ receptor ($InsP_3R$), which is itself a Ca^{2+} permeable ion channel, and the release of Ca^{2+} from intracellular endoplasmic reticulum (ER) Ca^{2+} stores. Chemokine receptors expressed on lymphocytes, which are, for example, responsible for lymphocyte migration to secondary lymphoid organs, also use PLC-mediated $InsP_3$ production to induce Ca^{2+} mobilization via the activation of PLCβ. Ca^{2+} released from stores results in only a moderate and transient increase in intracellular Ca^{2+} concentration because of the small size of the ER in lymphocytes. Importantly, the decrease of the Ca^{2+} concentration in the ER triggers the activation of a 'capacitative' or SOCE pathway, resulting in the opening of store-operated Ca^{2+} channels in the plasma membrane. In lymphocytes, the CRAC channel is the best described and main store-operated Ca^{2+} channel (BOX 1).

Patch clamp
An electrophysiological technique to study ion channel currents in living cells. The electrode is a glass pipette, which forms a tight seal with the plasma membrane. Calcium-release-activated calcium channel currents are typically recorded in whole cell configuration from the entire cell.

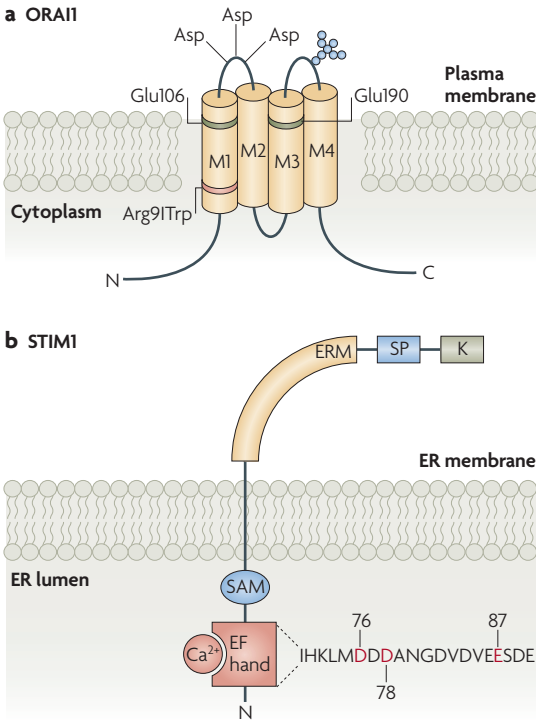


Figure 2 | **Domain architecture of the CRAC channel subunit ORAI1 and the Ca^{2+} sensor in the endoplasmic reticulum STIM1. a** | ORAI1 is a plasma membrane protein with four predicted transmembrane segments (M1–M4) and intracellular amino- and carboxy-termini[13,78–80]. The site of the Arg91Trp mutation in human patients with severe combined immunodeficiency (SCID)[13] that abrogates calcium-release-activated calcium (CRAC) channel function is indicated. Two conserved glutamate residues (Glu106 and Glu190) and three aspartate residues (Asp) implicated in the Ca^{2+} permeability of the CRAC channel are also shown[80,83,84]. **b** | Stromal interaction molecule 1 (STIM1) is a single-pass transmembrane protein that is mainly localized in the endoplasmic reticulum (ER). The N-terminus contains a Ca^{2+}-binding EF-hand motif with negatively charged residues (D76, D78 and E87) that are crucial for sensing Ca^{2+} (REFS 64,68,69). Predicted protein–protein interaction domains in STIM1 include a sterile α-motif (SAM), a coiled-coil ezrin, radixin and moesin (ERM) domain, a serine- and proline-rich region (SP) and a lysine-rich region (K).

SOCE and CRAC channels are present in many cell types of the immune system, including T cells, B cells, DCs and mast cells, and it is arguably the most important mechanism in these cell types for generating Ca^{2+} signals[1,47–50]. Ca^{2+} influx via CRAC channels in turn results in the sustained Ca^{2+} signal necessary for cytokine gene transcription, for example.

Ca^{2+} influx channels in T cells. Several Ca^{2+} influx pathways and channels have been proposed to exist in lymphocytes (TABLE 1). Most experimental evidence, however, points to a predominant role of CRAC channels in both T and B cells (BOX 1, TABLE 1). This notion is supported by the severe defect in SOCE and lymphocyte activation in the absence of functional CRAC channels in T cells (and to a lesser degree in B cells) from immunodeficient patients[4–6]. CRAC channels have been carefully studied and their biophysical properties are well defined, allowing for their unequivocal identification in lymphocytes despite the fact that their molecular identity remained a mystery for almost two decades[1–3] (BOX 1). Much attention has focused on the role of transient receptor potential (TRP) channels in SOCE and as possible candidate genes encoding the CRAC channel[51–53]. However, none of the TRP channels has unambiguously been shown to be activated by store depletion or have the biophysical properties of the CRAC channel. It therefore remains uncertain at the moment which role, if any, these channels play in SOCE[2,54]. In addition to SOCE and CRAC channels, other Ca^{2+} release and influx pathways have been described in both T and B cells, including L-type voltage-gated Ca^{2+} channels and ATP-binding, Ca^{2+} permeable P2X receptors (TABLE 1). Genetic modulation of the expression or function of regulatory voltage-gated Ca^{2+} channel subunits[55] and $P2X_7$ (REFS 56–58), respectively, was shown to attenuate Ca^{2+} signals in T cells, and it was suggested that these proteins participate in regulating intracellular Ca^{2+} levels. However, the presence of functional voltage-gated Ca^{2+} channels in T cells has not been demonstrated through recording of Ca^{2+} channel currents, and therefore their role remains unclear.

STIM1: sensor of ER Ca^{2+} levels and activator of CRAC. One of the mysteries of SOCE has been the mechanism by which depletion of ER stores regulates CRAC channel activation. At least three models were proposed, postulating 'conformational coupling'[59], a soluble Ca^{2+} influx factor[60] and vesicle fusion[61,62] as underlying SOCE, but none of these models has conclusively been proved (reviewed in REFS 2,3). Our understanding of the mechanism regulating SOCE was dramatically changed with the recent identification of STIM in two independent RNA interference (RNAi) screens[63,64]. RNAi-mediated knockdown of expression of Stim in *Drosophila* and of its homologue, STIM1, in human cells, significantly reduced SOCE. Earlier studies had identified STIM1 as a protein promoting survival of pre-B cells[65] and had linked it to a region on human chromosome 11p15 that is associated with the pathogenesis of rhabdomyosarcoma[66]. *Drosophila* Stim and STIM1 are single-transmembrane proteins localized in the ER and the plasma membrane[67] (FIG. 2). A Ca^{2+}-binding EF-hand motif is localized in the portion of STIM1 facing the ER lumen and STIM proteins were therefore postulated to act as sensors of Ca^{2+} concentration in the ER. Indeed, mutations in one or several Ca^{2+}-binding glutamate and aspartate residues within the EF-hand motif constitutively activated SOCE and CRAC channels independent of the filling state of the Ca^{2+} stores[64,68,69] (FIG. 2).

The exact mechanism by which STIM proteins activate CRAC channels in the plasma membrane is not fully understood. Store depletion, however, is associated with the relocalization of STIM1 into discrete 'puncta', which can be observed by confocal microscopy and which represent clusters of STIM1 proteins[64]. STIM1

RNA interference
(RNAi). The use of double-stranded RNAs with sequences that precisely match a given gene, to 'knockdown' the expression of that gene by directing RNA-degrading enzymes to destroy the encoded mRNA transcript. RNAi is involved in innate immune responses, as well as in organ development, and has been exploited in large scale screens for genes regulating certain aspects of cell function.

has indeed been shown to form homomultimers and heteromultimers with itself and STIM2 (REF. 67), and these interactions are mediated by one or several of the protein–protein interaction domains of STIM1 including a sterile α-motif (SAM), a coiled-coil ezrin, radixin and moesin (ERM) domain, a serine- and proline-rich region and a lysine-rich region[70] (FIG. 2). Oligomerization precedes the formation of STIM1 puncta, which are the result of local redistribution of STIM1 in the ER membrane[71]. Similar STIM1 clusters have been observed in non-stimulated cells expressing STIM1 proteins with mutant EF-hand motifs, indicating that the redistribution of STIM1 is indeed caused by dissociation of Ca^{2+} from the EF-hand motif[64]. These puncta are located in or near the plasma membrane, suggesting that STIM1 may interact, directly or indirectly, with CRAC channels[64,68], an idea that is further supported by the finding that STIM1 puncta co-localize with sites of Ca^{2+} influx and the CRAC channel subunit ORAI1 (REF. 72). As STIM1 was found to be expressed in the plasma membrane[73,74], it has been speculated whether it acts in *cis* or in *trans* on CRAC channels — that is, whether STIM1 clusters have to be present in the plasma membrane itself or in parts of the ER that are juxtaposed to the plasma membrane. Although incubation of cells with antibodies directed against the extracellular amino-terminal domain of STIM1 was able to impair SOCE[69], elegant electron-microscopy studies showed that Ca^{2+} store depletion results in the concentration of horseradish-peroxidase-tagged STIM1 in parts of the ER that are apposed to the plasma membrane but not in the plasma membrane itself[75].

Whereas STIM1 is unambiguously required for SOCE, the role of its paralogue STIM2 is not clearly defined. Although STIM2 and STIM1 show a high degree of sequence conservation, especially in their functional domains[76], knockdown of STIM2 expression resulted in either no effect or a more moderate effect than the knockdown of STIM1 expression on Ca^{2+} influx in HeLa cells[64] or human embryonic kidney 293 (HEK293) cells[63] (A. Iuga and S.F., unpublished observations). Whether STIM2 has a similar role in SOCE to STIM1 but only in certain cell types or has inhibitory effects on SOCE will have to be carefully analysed[77].

ORAI is a pore-forming subunit of the CRAC channel. Recent evidence from several laboratories suggests that the CRAC channel is encoded by a new, hitherto unrecognized gene, which is unrelated to TRP or any other known ion channel. Three independent genome-wide RNAi screens in *Drosophila* S2 cells found that depletion of the hypothetical gene product olf186-F abrogated Ca^{2+} influx[13,78,79]. Similarly, RNAi-mediated knockdown of FLJ14466, one of the three human homologues of olf186-F, strongly interfered with SOCE and CRAC channel function[79]. In humans, FLJ14466 is located on chromosome 12q24 and one of the studies that identified olf186-F had used positional cloning to link this region to a particular form of severe combined immunodeficiency (SCID) disease characterized by the absence of SOCE and CRAC channel currents[6,13]. Homozygosity for a missense mutation in FLJ14466 resulting in an arginine to tryptophan substitution at position 91 (Arg91Trp) of the protein abrogates the CRAC channel current in T cells derived from individuals with SCID. Overexpression of wild-type FLJ14466 in T cells from SCID patients reconstituted SOCE and CRAC channel currents with biophysical properties closely resembling those reported for the native CRAC channel[13]. Given the parallelism of its discovery and its causal relationship to human disease, FLJ14466 is widely accepted as an essential component of the SOCE pathway. *Drosophila* olf186-F was renamed *Drosophila* Orai and human FLJ14466 is now referred to as ORAI1 (REF. 13) or as CRACM1 by Vig *et al.*[79]

One of the intriguing questions following the discovery of ORAI1 was whether it is part of the CRAC channel itself or a protein that facilitates the opening of CRAC channels, presumably by binding to the channel. Hydrophobicity analysis of *Drosophila* Orai and human ORAI1 had predicted a transmembrane protein with four membrane domains (referred to as M1–M4) and intracellular N- and C-termini (FIG. 2). This topology, which is compatible with that of an ion channel gated by an intracellular ligand (STIM1 following store depletion), was confirmed by immunocytochemical and cell-surface biotinylation experiments[13,79,80]. To demonstrate that *Drosophila* Orai and human ORAI1 are part of the CRAC channel pore itself, several groups used site-directed mutagenesis of conserved glutamates in the first and third predicted transmembrane domains of *Drosophila* Orai and human ORAI1. Negatively charged glutamates are known to provide Ca^{2+} binding sites in various Ca^{2+} channels, including voltage-gated Ca^{2+} channels and certain TRP channels, and are part of their Ca^{2+} selectivity filter[81,82]. Mutation of Glu106 in human ORAI1 and the corresponding Glu178 (described as Glu180 in REF. 83) in *Drosophila* Orai greatly reduced the Ca^{2+} selectivity of the CRAC channel current, and rendered the mutated channel, unlike native CRAC channels, permeable to Na^+ and Cs^+ even in the presence of external Ca^{2+} (REFS 80,83,84). These findings indicated that *Drosophila* Orai and human ORAI1 are crucial for ion conductance and Ca^{2+} selectivity of the CRAC channel and therefore likely to be pore-forming subunits of the long elusive CRAC channel.

ORAI1 shares its tetraspanning protein topology with two structurally related paralogues, ORAI2 (also known as CRACM2 or TMEM142B; the gene of which is located on chromosome 7q22.1) and ORAI3 (also known as CRACM3 or TMEM142C; the gene of which is located on chromosome 16p11.2). Overexpression of ORAI3, but not ORAI2, in ORAI1-deficient T cells partially restored Ca^{2+} influx[85] and combined expression of ORAI1 or ORAI2 (but not ORAI3) with STIM1 dramatically enhanced Ca^{2+} influx in HEK293 cells[86]. These data indicate that ORAI2 and ORAI3 may themselves form Ca^{2+} permeable ion channels. This idea is supported by the finding that overexpression of ORAI2 and ORAI3 in HEK293 cells, respectively, gives rise to currents with properties that are distinct from those of bona fide CRAC channels[87,88].

Severe combined immunodeficiency (SCID). A primary (inherited) immunodeficiency characterized by defects in cell-mediated and humoral immune responses. Affected infants commonly die within 1 year due to recurrent infections. Mutations in about 10 different genes have been described, but defects in the common cytokine-receptor γ-chain are the most common form causing X-linked SCID. Other genes mutated in SCID include Janus kinase 3 (*JAK3*), recombination activating gene 1 (*RAG1*) and *RAG2*, IL-7 receptor α-chain (*IL7R*) and adenosine deaminase (*ADA*).

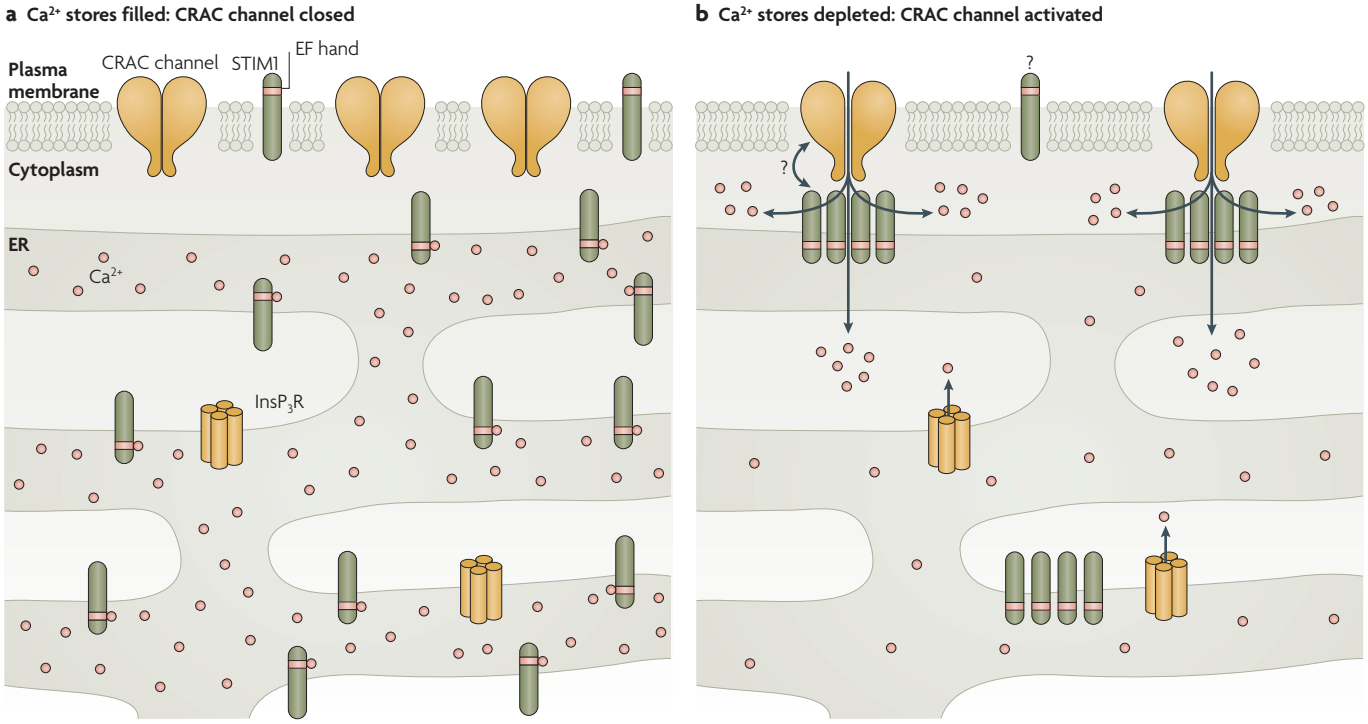


Figure 3 | **Redistribution of STIM1 following Ca^{2+} store depletion.** In resting cells with filled endoplasmic reticulum (ER) Ca^{2+} stores, most stromal interaction molecules 1 (STIM1s) are localized in the ER with an apparently homogeneous distribution pattern (**a**). Under these conditions, the EF-hand motif of STIM1 is bound by Ca^{2+} ions and calcium-release-activated calcium (CRAC) channels are closed. Upon depletion of ER Ca^{2+} stores and dissociation of Ca^{2+} from the EF-hand motif, STIM1 redistributes into discrete puncta[64] (**b**). A fraction of these puncta is localized close to the plasma membrane[75] and co-localizes with clusters of ORAI1-containing CRAC channels and sites of localized Ca^{2+} influx in the plasma membrane[72]. Although STIM1 was described to be localized in the plasma membrane in addition to the ER[68,69], it is not clear whether this STIM1 fraction is involved in CRAC channel activation. Currently available data favour a model in which clusters of STIM1 in the ER act in *trans* on the CRAC channel localized in the plasma membrane.

With the discovery of STIM1 and ORAI1 in the past 2 years two key molecules in SOCE have been identified. That both proteins indeed operate in the same pathway is strongly suggested by experiments in which co-expression of Stim and Orai in *Drosophila* S2 cells and STIM1 and ORAI1 in HEK293, RBL (rat basophilic leukaemia) and Jurkat T cells, resulted in a dramatic increase in CRAC channel currents[83,89,90]. In the simplest model derived from these studies, STIM1 functions as the sensor of Ca^{2+} concentration in the ER and the activator of ORAI1 CRAC channels in the plasma membrane; both proteins appear to be the limiting components of this pathway (FIG. 3). Further investigations are required to understand whether STIM1 and ORAI1 directly interact with each other[83,85] and what role STIM2 has in this process. Important gaps in our understanding of SOCE function include clarification of the precise molecular composition of the CRAC channel, whether it is a homomultimer or heteromultimer, the stochiometry of ORAI1 in this complex and whether ORAI2 and ORAI3 are part of the CRAC channel complex.

Other mechanisms regulating Ca^{2+} levels in lymphocytes. As mentioned, T and B cells use various mechanisms to regulate intracellular Ca^{2+} levels, some of which are lymphocyte specific and others which are shared with other cell types. Influx and efflux of Ca^{2+} across the plasma membrane is modulated by common mechanisms such as K^+ channels, plasma membrane Ca^{2+} pumps and mitochondrial Ca^{2+} uptake, as detailed in FIG. 4. B cells have, in addition, developed specific mechanisms, which allow them to enhance or dampen the Ca^{2+} signal emanating from BCR activation (TABLE 1). Signals resulting from CD19 engagement synergize with BCR signals to enhance Ca^{2+} influx[91], and B cells from CD19-deficient mice or human subjects with mutations in CD19 have reduced Ca^{2+} influx[92,93]. By contrast, Ca^{2+} responses in B cells are counterbalanced by a series of negative regulators, which target the activity of PI3K (phosphoinositide 3-kinase), PLCγ2 and hence the level of $InsP_3$; among those molecules are CD22 (REFS 94–96), paired immunoglobulin-like receptor B (PIRB)[97,98] and FcγRIIB (REF. 99). PIRB is linked to SH2-domain-containing protein tyrosine phosphatase 1 (SHP1) and SHP2, and and FcγRIIB is linked to SH2-domain-containing inositol 5′ phosphatase (SHIP)[97,100]. The Ca^{2+} influx in response to BCR ligation in mice lacking any of these negative regulators is enhanced, thus, potentially contributing to the autoimmune phenotypes observed in these mice[96,101,102].

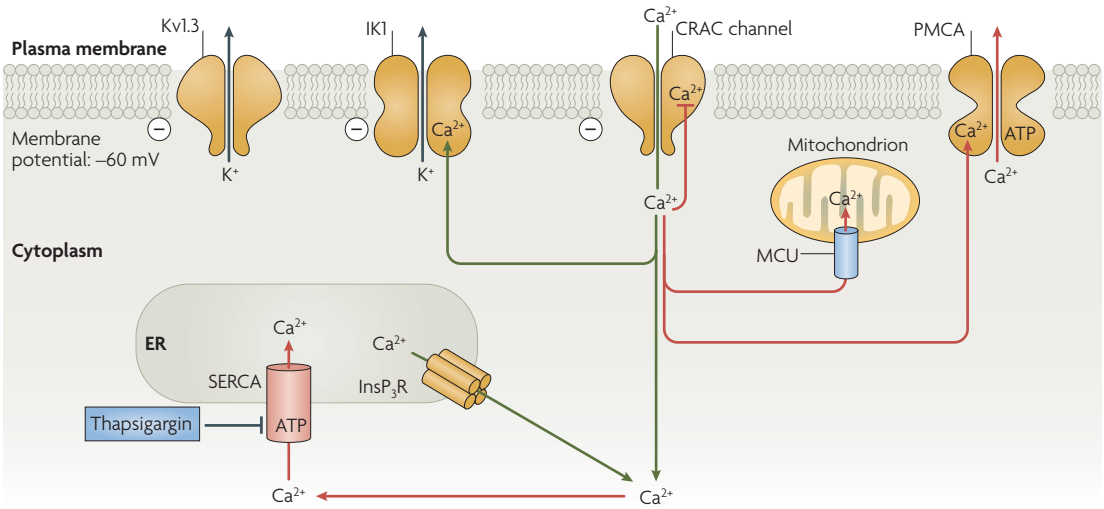


Figure 4 | **Mechanisms regulating Ca^{2+} levels in T cells.** Several mechanisms influence the shape, duration and strength of the Ca^{2+} signal. In T cells, two types of K^+ channel generate a negative membrane potential (approximately –60 to –70 mV) that is required for Ca^{2+} influx through calcium-release-activated calcium (CRAC) channels[131,132]. Depolarization of the cells reduces the driving force for Ca^{2+} entry, a process that is counteracted by the opening of Kv1.3 channels. IK1 (intermediate conductance calcium-activated potassium channel protein 1) channels, by contrast, open in response to Ca^{2+} influx and increased intracellular Ca^{2+} concentration. Ca^{2+} signals are attenuated by excluding Ca^{2+} from the cytoplasm with the help of ATP-dependent Ca^{2+} pumps in the endoplasmic reticulum (ER; such as sarco/endoplasmic reticulum Ca^{2+} ATPase, SERCA) and plasma membrane (such as plasma membrane Ca^{2+} ATPase (PMCA)). The SERCA pump is the target of inhibitory drugs, such as thapsigargin, which are frequently used to induce Ca^{2+} store depletion and thereby CRAC channel activation. PMCAs are activated by increases in intracellular Ca^{2+} concentration and are crucial for attenuation of the initial peak Ca^{2+} influx[133]. Mitochondria have a dual role in shaping the Ca^{2+} signal: they take up and store large amounts of Ca^{2+} from the cytoplasm using a mitochondrial Ca^{2+} uniporter (MCU) located in the mitochondrial inner membrane[134]. In close proximity to CRAC channels in the plasma membrane, mitochondria sequester Ca^{2+} locally and prevent Ca^{2+}-dependent negative feedback inhibition of CRAC channels[135]. Pathways enhancing and dampening intracellular Ca^{2+} levels are shown in green and red, respectively. $InsP_3R$, inositol-1,4,5-trisphosphate receptor.

Lymphocyte Ca^{2+} signals and disease

Ca^{2+} signals in congenital immunodeficiency syndromes. In the immune system, impaired Ca^{2+} signalling in T and B cells has been linked to several inherited immunodeficiency diseases, which although rare have greatly contributed to our understanding of the *in vivo* role of Ca^{2+} signals in the immune response.

SCID. The essential CRAC channel subunit ORAI1 was identified by studying a small cohort of patients with SCID characterized by defective SOCE in their T cells. Positional cloning in these patients and their relatives linked their phenotype to chromosome 12q24, and, together with a genome-wide RNAi screen, led to the discovery of ORAI1 (REF. 13). A missense mutation and Arg91Trp substitution in the first transmembrane domain of ORAI1 is responsible for completely abrogating CRAC channel current activity in T cells from these SCID patients. A similar defect in SOCE and CRAC channel function was observed in two unrelated patients with SCID, but their molecular defects have not yet been described[4,5]. SCID is often associated with impaired development of T, B and NK cells (reviewed in REFS 103,104). In infants with SCID resulting from mutations in ORAI1, peripheral T and B cells were present at normal numbers[11]; it therefore seems that the function of ORAI1 is dispensable for lymphocyte development. The lack of SOCE in these SCID patients was, however, associated with severely compromised T-cell proliferation, expression of cytokines, including IL-2, IL-4, IL-10, IFNγ and tumour-necrosis factor (TNF), and antibody responses to T-cell-dependent antigens[4,5,11]. The functional defect in SCID patients is not limited to T cells and also affects SOCE in B cells[5,12] and fibroblasts[5,12], as well as in the neutrophils and platelets of one of the SCID patients whose molecular defect has not yet been characterized[5]. The Arg91Trp substitution in ORAI1 results, in addition to SCID, in mild extra-immunological symptoms in the form of non-progressive muscular dysplasia and hypohydrotic ectodermal dysplasia (HED), pointing to a potential role for ORAI1 in skeletal muscle and ectodermally derived tissues such as teeth and sweat glands (S.F. *et al.*, unpublished observations). However, when taking all these results in conjunction, it would seem that T cells are particularly dependent on the ORAI1 pathway for proper activation. In the future, studying other immunodeficient patients with defects in Ca^{2+} signalling might lead to further mechanistic insights into the regulation of SOCE and other Ca^{2+} signalling pathways.

B-cell immunodeficiencies associated with defects in Ca^{2+} signalling. X-linked agammaglobulinaemia (XLA) is an inherited B-cell immunodeficiency characterized by a defect in B-cell development, low numbers of mature B cells and a lack of immunoglobulin production[105]. The disease is caused by mutations in Bruton's tyrosine kinase (BTK), a non-receptor tyrosine kinase of the TEC family that enhances PLCγ2 activation and is required for sustained production of $InsP_3$ and Ca^{2+} mobilization. B cells from patients with mutations in BTK and *Btk*$^{-/-}$ mice show strongly impaired Ca^{2+} influx upon BCR crosslinking[106,107] (reviewed in REF. 105). In T cells, the TEC kinase ITK has a similar role to BTK in B cells, and T cells from *Itk*$^{-/-}$ mice have severely reduced SOCE, which contributes to the functional T-cell defect in these mice[108].

Similar to XLA, common variable immunodeficiency (CVID) is characterized by hypogammaglobulinaemia and recurrent bacterial infections in affected patients, but, unlike in XLA, B cells are present at near-normal numbers in individuals with CVID but these fail to mature into immunoglobulin-producing cells. The genetic causes of CVID are heterogenous but a subset of cases are caused by mutations in cell-surface receptors required for late B-cell maturation and immunoglobulin class switching (reviewed in REF. 109). One of the molecules mutated in CVID is CD19, the function of which is to lower the threshold of BCR signalling[110], and its crosslinking results in an increase in intracellular Ca^{2+} concentration. In turn, B cells from patients with CVID and mutations in CD19 have impaired Ca^{2+} influx, compromised B-cell responses to antigen and reduced numbers of $CD27^+$ memory B cells[93]. Similar observations were made in *Cd19*$^{-/-}$ mice, including the defects in immunoglobulin class switching, production of high-affinity antibodies and memory B cells[111,112]. In addition to B-cell-intrinsic defects leading to CVID, impaired T-cell help to B cells can also cause CVID, and defects in T-cell activation associated with reduced Ca^{2+} influx have been described in some cases of CVID[113].

Wiskott–Aldrich syndrome (WAS). The relationship between Ca^{2+} signalling and the actin cytoskeleton in T cells is a matter of debate[18,114]. It is therefore interesting to note that patients with mutations in the gene encoding Wiskott–Aldrich syndrome protein (WASP), a key regulatory protein of F-actin assembly, show impaired T-cell signalling characterized by, among other defects, reduced Ca^{2+} influx and reduced activation of NFAT, which results in reduced proliferation and IL-2 production[115,116]. Similar defects have been observed in NK cells from patients with WAS and in T cells from WASP-deficient mice[117]. That actin assembly may be involved in the activation of CRAC channels was also suggested by recent findings showing that knockdown of expression of the WASP homologue WAVE2 in T cells diminishes Ca^{2+} influx downstream of store depletion[118].

Ca^{2+} signals in autoimmunity and inflammation

Dysregulated Ca^{2+} responses have been associated with the pathophysiological processes in several autoimmune and inflammatory diseases. In systemic lupus erythematosus (SLE), an autoimmune inflammatory syndrome characterized by autoantibody production among other features[119], several lines of evidence suggest that signalling through the BCR in B cells is abnormal and results in increased Ca^{2+} signals. First, stimulation of B cells from patients with SLE with antibodies against surface IgM showed increased intracellular Ca^{2+} concentration compared with samples from healthy controls[120]. Second, increased Ca^{2+} influx in B cells from patients with SLE was correlated with impaired FcγRIIB signalling and reduced expression levels of SHIP[121]. FcγRIIB normally attenuates Ca^{2+} signals through the activation of SHIP and hydrolysis of phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$), and by inhibiting CD19-mediated Ca^{2+} responses[122]. Third, FcγRIIB-deficient mice spontaneously developed autoantibodies and glomerulonephritis, an effect that was observed, however, only on a particular genetic background, arguing that FcγRIIB constitutes a susceptibility factor for the development of autoimmune disease[101]. Fourth, mice deficient for other inhibitors of BCR signalling and Ca^{2+} responses in B cells, such as SHP1 and LYN, also developed autoimmune disease[123,124], and the absence of CD22 led to autoantibody production and increased Ca^{2+} signalling responses in B cells[96,102]. And fifth, mice with a gain-of-function mutation in PLCγ2 were shown to develop multiorgan autoimmune inflammatory disease with increased Ca^{2+} responses in B cells and presumably other cell types, such as granulocytes, because the disease phenotype persisted in the absence of B cells[125]. Taken together, these data support the idea that abnormal B-cell signalling and increased Ca^{2+} responses may be involved in the breakdown of B-cell tolerance and autoimmunity in SLE. Although intriguing, the evidence is mostly circumstantial, and the mechanisms leading to increased B-cell responses, the stage of B-cell differentiation at which they occur and, most importantly, whether increased Ca^{2+} signals are causally related to the pathogenesis of autoimmunity remain uncertain at this point.

Autoreactive T cells have an important role in the development of several autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis and type 1 diabetes. Similar to the development of autoreactive B cells in SLE, it is unclear why T cells become autoreactive in patients with, for example, rheumatoid arthritis, even though failed negative selection of T cells in the thymus has been implicated in the pathogenesis of rheumatoid arthritis. This hypothesis that failed negative selection is involved in the development of autoimmunity is supported by an *in vivo* model of rheumatoid arthritis, in which mutation of the kinase ZAP70 (ζ-chain-associated protein kinase of 70 kDa) results in strongly reduced (but not abrogated) TCR signals including Ca^{2+} signalling[126]. The altered signal strength, it is argued, changes the threshold of T-cell selection in the thymus, allowing T cells with self-reactive TCRs to escape negative selection and become arthritogenic in the periphery. Ca^{2+} signals are likely to participate in the fine-tuning of TCR signals in thymocytes, and hence in the selection of functional T cells and the depletion of autoreactive T cells.

TEC family
A family of non-receptor protein tyrosine kinases that contain a pleckstrin-homology domain. The prototype members are ITK (interleukin-2-inducible T-cell kinase) in T cells and BTK (Bruton's tyrosine kinase) in B cells. TEC-family kinases are involved in the intracellular signalling mechanisms of cytokine receptors, lymphocyte antigen receptors, heterotrimeric G-protein-coupled receptors and integrins.

Immunoglobulin class switching
A region-specific recombination process that occurs in antigen-activated B cells. This occurs between switch-region DNA sequences and results in a change in the class of antibody that is produced — from IgM to either IgG, IgA or IgE. This imparts flexibility to the humoral immune response and allows it to exploit the different capacities of these antibody classes to activate the appropriate downstream effector mechanisms.

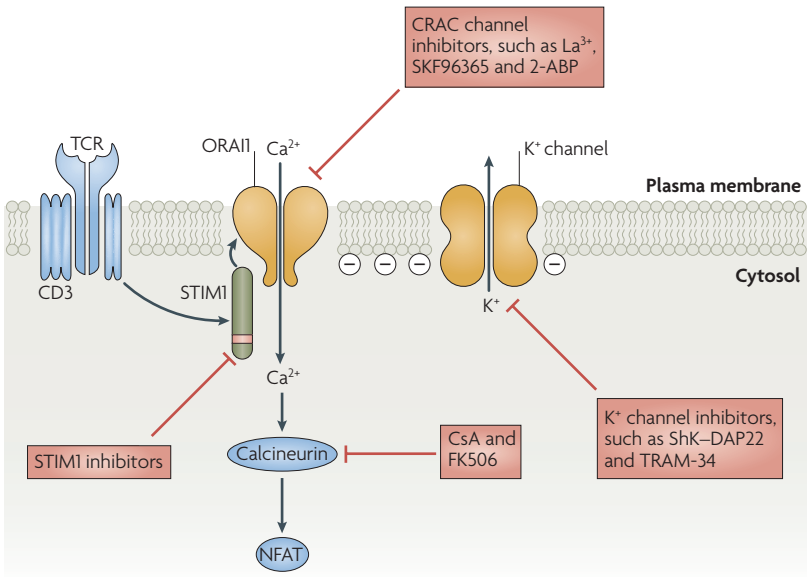


Figure 5 | **The CRAC-channel–calcium–calcineurin–NFAT signalling pathway as a target for therapeutic immune modulation.** Molecular targets in this pathway are the calcium-release-activated calcium (CRAC) channel subunit ORAI1, the CRAC channel regulatory protein stromal interaction molecule 1 (STIM1), the K^+ channels Kv1.3 and IK1 (intermediate conductance calcium-activated potassium channel protein 1), which provide the negative membrane potential necessary for Ca^{2+} influx, the phosphatase calcineurin and the transcription factor NFAT (nuclear factor of activated T cells). Small peptide inhibitors of Kv1.3 and IK1, ShK–DAP22 and TRAM-34, respectively, have been shown to ameliorate the outcome of experimental autoimmune encephalomyelitis[127,128]. The calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK506) potently block T-cell activation and are in clinical use to prevent transplant rejection and to treat severe forms of autoimmune disease[136]. No specific inhibitors of CRAC channel activation have been published, although several compounds are frequently used *in vitro* to block store-operated calcium entry (SOCE) and CRAC channel currents, including the trivalent cations La^{3+} and Gd^{3+}, and the small-molecule blockers SKF96365 and 2-APB[49]. TCR, T-cell receptor.

Therapeutic potential

The involvement of dysregulated lymphocyte Ca^{2+} signalling in the pathogenesis of autoimmune and inflammatory immune disorders suggests that interference with Ca^{2+} signalling in general and SOCE through the ORAI1–STIM1 pathway in particular may be a useful approach to treat autoimmune diseases, such as SLE or rheumatoid arthritis, and inflammatory disorders, such as psoriasis or ulcerative colitis, and to prevent allograft rejection in transplantation (FIG. 5). Several lines of evidence support this idea. First, inhibition of the Ca^{2+}-dependent phosphatase calcineurin with cyclosporin A and FK506 is an established therapeutic approach to avert the rejection of allogeneic organ transplants and for the treatment of more severe cases of rheumatoid arthritis, psoriasis or ulcerative colitis. Because the transcriptional responses in T cells downstream of Ca^{2+} and calcineurin signals overlap significantly, as discussed earlier[12,26,27], it can be expected that the inhibition of CRAC-channel-mediated Ca^{2+} signals has a similar immunomodulatory potential as cyclosporin A and FK506. Second, interfering with SOCE indirectly via pharmacological inhibition of K^+ channels in myelin basic protein (MBP)-specific T cells significantly ameliorated disease outcome in experimental autoimmune encephalitis (EAE; an animal model of multiple sclerosis)[127,128]. Inhibition of the voltage-gated K^+ channels Kv1.3 and IK1 (intermediate conductance calcium-activated potassium channel protein 1) with ShK–DAP22, a small peptide inhibitor derived from the sea anemone *Stichodactyla helianthus*, and TRAM-34 (1-[(2-chlorophenyl)diphenylmethyl]-1*H*-pyrazole), respectively, ameliorated the course of EAE in adoptive transfer models in rats and mice[127,128]. Inhibition of Ca^{2+} influx through K^+ channels may therefore provide a means to modulate T-cell activation in disease. Third, patients with SCID lacking CRAC-channel-mediated Ca^{2+} signals due to a mutation in *ORAI1* show a severe attenuation of T-cell activation that seems to be rather specific to this cell type, whereas non-immunological symptoms are relatively mild, as discussed earlier[6,11,13,129] (S.F. *et al.*, unpublished observations). There are currently no specific inhibitors of SOCE and CRAC channels available to test the benefits of blocking this pathway *in vivo* in a disease model (FIG. 5). Specific inhibitors of ORAI1 and CRAC channel function might have fewer unwanted adverse effects than cyclosporin A or FK506, because calcineurin function is crucial in many cell types and organs, whereas ORAI1 may have non-redundant functions only in a few cell types, including T cells, as judged by the phenotype in SCID patients[130]. The chances of developing such inhibitors should improve now that the first two molecules in this pathway, STIM1 and ORAI1, have been identified.

Concluding remarks

The past 2 years have seen remarkable progress in our understanding of the molecular nature and the regulation of the most important and hitherto elusive Ca^{2+} signalling pathway in lymphocytes — SOCE through CRAC channels. With the identification of ORAI1 as a subunit of the CRAC channel and STIM1 as an essential regulator of channel opening, we now have the tools to study in greater detail the molecular composition and stoichiometry of the CRAC channel, its gating mechanisms, its interactions with other molecules and other putative channel subunits. With these two cornerstones in hand, we can try to delineate the SOCE pathway as a whole, not only in lymphocytes but also in other cell types. It will now be possible to investigate the role of ORAI1, STIM1 and their paralogues in SOCE in different tissues and organs *in vivo*. In particular, we can study the role of these molecules in T-cell- and B-cell-mediated immune responses *in vivo* and also, as SOCE has been described in various immune cells besides lymphocytes, in NK cells, DCs and mast cells. Ca^{2+} signals have been observed in immature T cells and were suggested to be involved in developmental progression and selection processes in thymocytes. By contrast, T-cell development does not seem to be impaired in human SCID patients who lack functional ORAI1 (REF. 11). This apparent contradiction could be explained by the fact that SOCE in thymocytes, in contrast to mature T cells,

is independent of ORAI1 and is mediated either by ORAI2 and ORAI3 or independent mechanisms. The respective roles of these molecules can now be examined *in vivo*. Modulation of T-cell and B-cell activation for the therapy of diseases of the immune system using inhibitors of SOCE is an exciting new possibility. The discovery of STIM1 and ORAI1 has increased the chances that such inhibitory drugs will be developed and will complement the armamentarium for the treatment of autoimmune and other diseases.

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Competing interests statement
The author declares competing financial interests: see web version for details.

DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
NFAT1 | ORAI1 | ORAI2 | ORAI3 | STIM1 | STIM2
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CVID | WAS | XLA

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